The Genomic Structure of the Mouse Csx/Nkx2-5

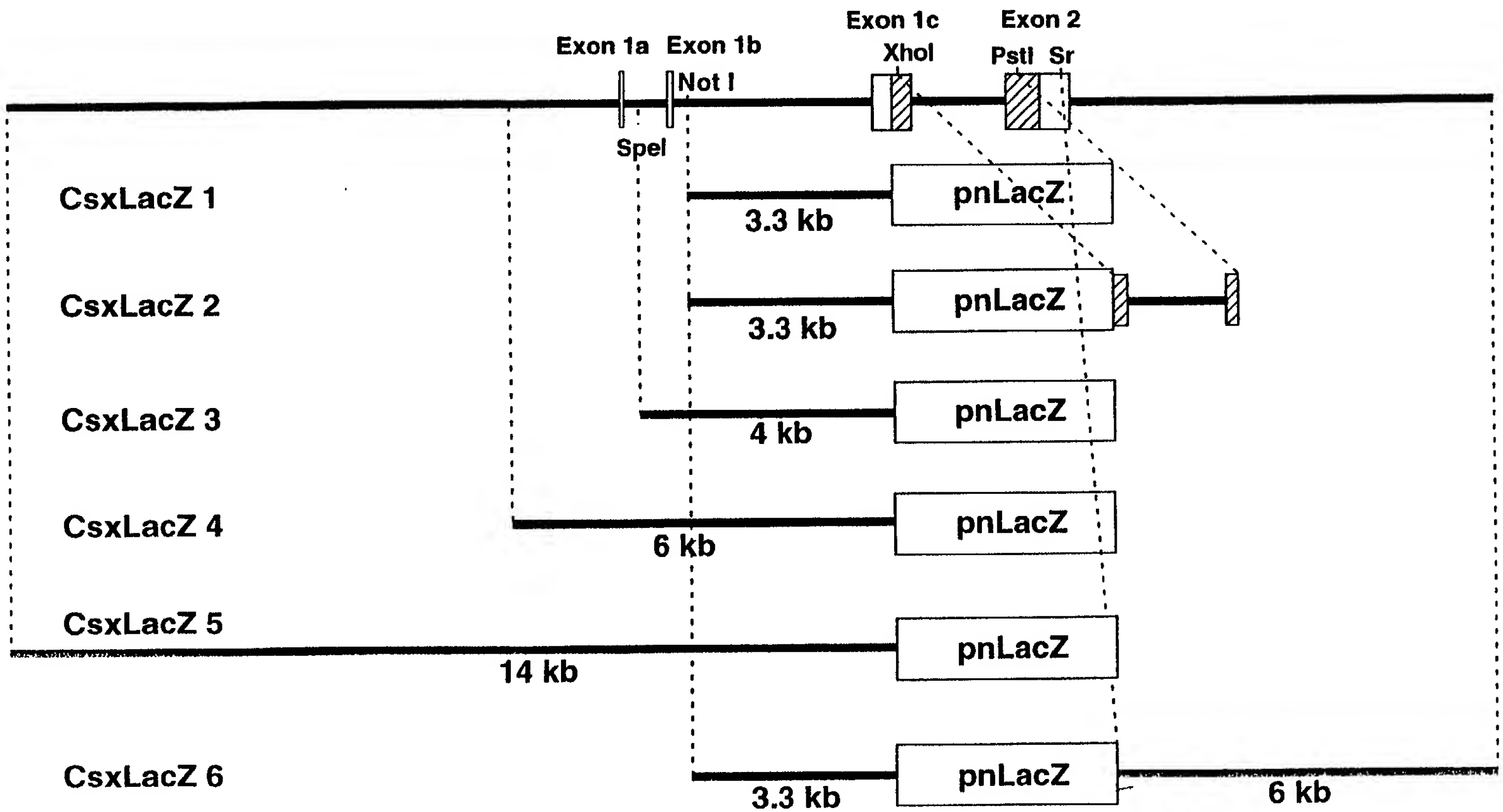


Fig. 1

The Locations of the Csx/Nkx2-5 Cardiac Enhancers

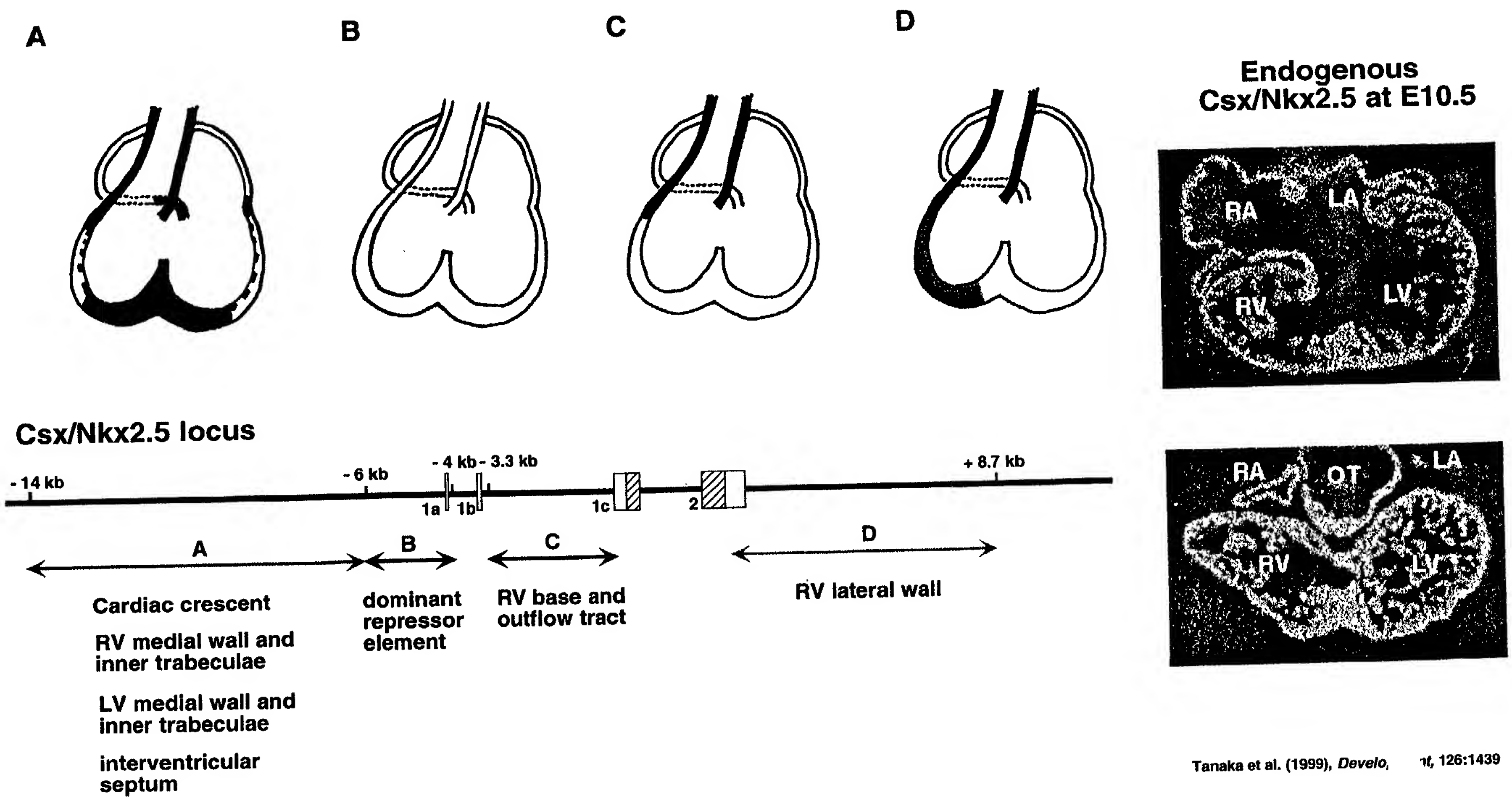
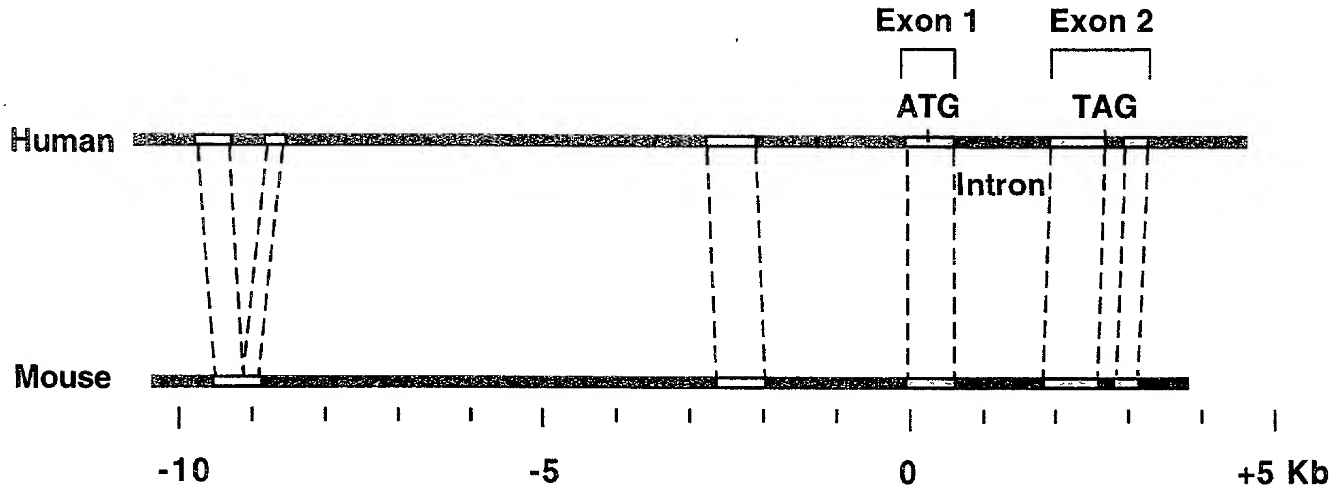


Fig. 2


NotI
ScaI
ScaI
XhoI
EcoRI
XhoI
HindIII
ScaI
XmnI
PvuI
HindIII
XhoI
XbaI
BlpI
BlpI
ATG
EcoRI
NotI
HindIII
Exon 1
Intron
Exon 2
-17 kb
-15
-10
-5
0
+5
+8 kb
20 kb
hsp68-lacZ
7.5 kb
hsp68-lacZ
1.4 kb
pnlacZ
3.5 kb
hsp68-lacZ
4 kb
hsp68-lacZ
1 kb
hsp68-lacZ
0.7 kb
hsp68-lacZ
0.3 kb
hsp68-lacZ
Human
Mouse
<Sp-1
Sp-1>
GATA>
<dHAND
TGF-b>
<MEF2
<GATA
Sp-1>
<Csx
CArG>
E-Box>
<Csx

Fig. 3A




```
Human   1    AGAGAAATCATTACCCGATTCACAAAGAGCATAGAGAGTGTAACAGTCACTGATCTTGTTCAAATAGGGAGAGTTTTTTTTCCTTCCCTTTTTGTA  96
Mouse   1   GAAGAGAAAGGCCAGGGCCTT-TTCACCAAGGTTGTAG-GGGTGGGAAAGTCACTGATTTTGTTCAAATTAGAAGAGTTTT-----CTTCCTTTTTCTTG  93
             *** *    **    **    ***** ***      *** * ***  * *********** **********   * ********       ***** ****   *

Human  97   ACACCTGACCCACAGGACTGACAGTTCTAGGAAGCCCCCTTACCCGAAAATAGGAAATAAATCCTTGCCACCTTGATTTGCAAGGGCAATGCTAATTTTT 196
Mouse  94   ACACCTGACCCACAGATTTGTCAACTCT-GGAAGCCCTTATATCCGAAAA-AAGTGATAAATCCTCACCACATTGATTTGCAAAGGAAACG--------- 182
            ***************   ** **   *** ********    ** ******* * *   *********  **** *********** ** ** *

Human 197   TTCTTTCTCCAGAGCTCTCAAAAAAAAAAAAAAAAAAAACCTTACTAAAAACAGGGATCCCGGATGTAGCCTCGATGTCCCCCATTAAACGGTAATATTTC 296
Mouse 183   -TCCCCTCTCAAAACTATTTTTTAAAAAAGACTTAAAACAGGGATCCCGGATGGGGGCCCCAATATTGCTCC------CCCAATTAAACGGTAATATTTC 275
             **        ** * ** *      ****** *   *****        *          ***  ***   ** * ** *     *** *****************

Human 297   AGGCGTCCGCTCACACTAATCTTTCAAACTGTCATCGCGAGCCGCCTGGCCAGCAGATTCACTT-AACAGCGCTCCCAGGACCCTCGTTCCGAGCTCTTT 395
Mouse 276   AGGCGTCAGCTCACACTAATCTTTCAAACTGTCATCGCGATCCGCAGGGGCAGCCGATTCACTTTAACAGGACTCCCAGGACC-TCGCCCGAAGCTGTTT 374
            ******* ******************************** ****  ** **** ********* ******  *********** ***   * **** ***

Human 396   TCAGCGAGACATTTAATTGAATCGGATGTGGCTCGTTTGCCAGACGTCACCGCCTCGGCGATAGGCATCCTCTCCAACGACACCCCCCCCGCCCGCGCT 495
Mouse 375   TCAGCAAGACATTTAATTGAATCGGATGTGGCTCGTTTGCCAGACGTCACCGCCTGGGTGATAGACATCCGTCCAACCCACACAAACCAATCTTCAATCT 474
            ***** ************************************************* ** ***** *****    * * * ****   **       *    **

Human 496   CGAAAACAATCTTCA  510
Mouse 475   CGGGAGGGTCCCAAGC 490
             **  *     *
```

```
Human   1 TAGGAAAAAAAAAAAAAAGGAAGCCCTCCGGGAGAGACATGCGCCCTAATATTTCTCCCAGATGGGCCGGGTTCAAGCGCGTTTGAGAGTTTGCTCTCCT  100
Mouse   1 CACACAGACAAGCAGTCAATGCGCTGTTTGGCTGTTCCTTGTGTT----TGTGGTTGAAAAGCGGTCCACACACACCCGT-TCTGAGTGACAGTGTGCC-   95
           *   * * **  *   *    **  *  **  *   * ** *      * *   *   *   ** **     **  **  * **** *   *   **

Human 101 ACCAGCCTCGGGTTCTAGGCCCCCCGCACCCTCATCCTGGCTCCCGCCCCTTCTCTCCACCCTCCCGGACCCCTAAAGGGGCGGCGGGGCCCAAGCCGAG 200
Mouse  96 -CCAGTGACCCGCTCCATGTCCCA-GAATCTTCATGCGGGCTGCAGCATCTCCTTCCCACCCTCCAGGAACGC--GAAGGACAGCGG------AGCCGAG 185
           ****   *  * ** * * ***  * * * **** * **** * **  ** **  ********* *** * *    * ** * ****      *******

Human 201 GGCGCTGCGCCTGACCCCGAGCGGAAGGGCCCCAGTCTAGGTCCTAATGCGGGTGGCGTCTCCTTTGACAGGCGGCGTTTGGGGACAACAGCGGGGACGA 300
Mouse 186 GGGGCAGCGCGTGACCCCGAGCGGAAGGGCCCCAGTCTGGGTCCTAATGCGGGTGGCGTCTCTCTTGACAGGCAGCGTTTGGGGACAACAGCGGGGAAGG 285
          ** ** **** ***************************** *********************** ********* ************************** *

            GATA>              <dHAND    TGF-b>                                               <MEF2
Human 301 GAGATAAGGTGACATACCAGAGCAGATTTGGTGCGCGCGCTGATACTCCTCTCCCGACAGGAAACGCGGAGCTATTTAAAA-GACCCTATCGATTACTTT 399
Mouse 286 GAGATAAGATGACATACCAGAGCAGATTTGGTGTGCGCGCTGATACTCCTGGCCCGACAGGAAACTCGGAGCTATTTAAAAAGGCCCTATCGATTACTTT 385
          ******** ************************ *************** ************** *************** * ****************

          <GATA
Human 400 ATCTTTCCTGGAAAGCTTCTTGCGGAGAGACAAAAGATGTTCCC-TGCCTAAAGACACAAGGCCACACAACGGAGGGTCTGCACAGGCGACGCACAATTC  498
Mouse 386 ATCTTCCCCGGAGGAAATCTTGCCGAGAGACAAAAGATGTCCCCCTACCTAAAGATACAAGGCCACACAGCAGAGGGTTTGTACAGGCGACGGGCGAGTA  485
          ***** ** ***      ****** ***************** *** * ********* ************* * ****** ** *********** * * *

Human 499 GGCGCGGGGAAAGCAAAAACACACTGACGCTTAGAGTGCACAAACGTGTGTGTTCCCAGAGCAGCTCCAGAGTGCGGCAGGGACGCTGGGGGCGGCGAGG  598
Mouse 486 GATCCCTGGGAGCGCAGAGACGCCTTTCTACCGGCAGAGACTGAAGTTTGACTGGAGCGAGGCGGGGCGGAGCCACCTCCCGGTCCCCGTGCACTCCGGA  585
          *   *  ** *     * *    ** *      *      ** * ** ** *     *** *   * ***            *   *    *    *  *

Human 599 GGCACCCACAGTATGGTCTTCTGTGCCCTTGGAAAGTTTTTTTTCACCGTATGCGCGTAAAACACGCACACACAGAGAAAGTGACTGTGCACTTAGGGCG  698
Mouse 586 ATTGTGAACGCTCCCGCAAAGTCCCCGCGAGTGTTGTGTTCACACTAATTGGTGTGCCTCTGAGTTCC-------------------------------   653
                   *  *    *      *  * *  *    ** **    *    *                *

.uman 699 CCTGTGTGTACCCGTGTCGTTTTAGCGAATTTAAAGCACATCAGGCCGGGCGCCATGGCTCACGCCTGTAATCCCAGCACTTTAGGAGGCCGAGGCGGGC  798
Mouse     ----------------------------------------------------------------------------------------------------

Human 799 CGATCACCTGAGGTCGGGAGTTCGACACCAGCCTGGCCAACATGGTGAAACCCTGTCTCTACAAAAAATACAAAAATTAGCCGGGCATGGTGATGCGTGC  898
Mouse     ----------------------------------------------------------------------------------------------------

Human 899 CTGTGATCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCACACCACTGCACTCCAGCCT  998
Mouse     ----------------------------------------------------------------------------------------------------

Human 999 GGGCGACAAGAGCGAAATTCCGTCTAAAAAAATAAAATAAAATAAAATGATAATTAAGCCCATCAACTCACATTCAAAGCGGTTACTGGTGGTTGTAATG 1098
Mouse     ----------------------------------------------------------------------------------------------------
```

Fig. 3B

The Genomic DNA Sequence Homology Between Human and Mouse Csx/Nkx2-5

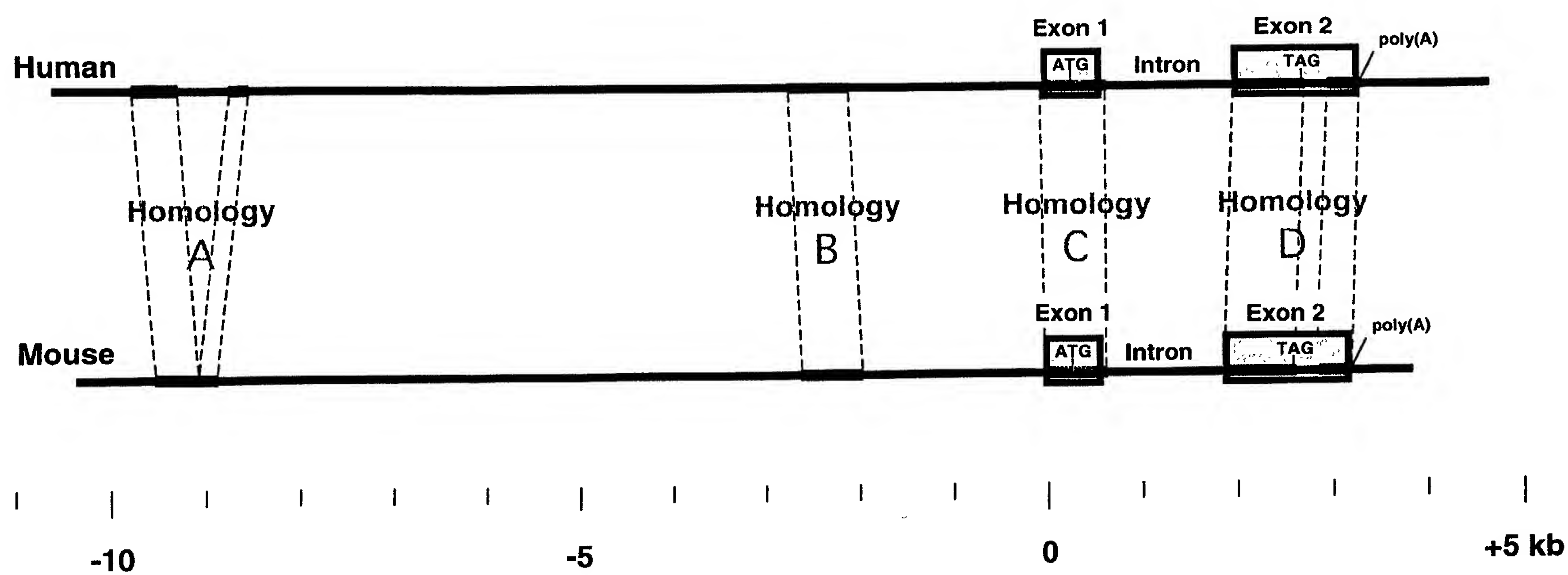


Fig. 3C

CTCGAGCCCAGGAGTTCAAGACCAGCCTGGGAAACATAGGGAGACCCC
TCTCTCTCCACAAAAAATTTAAAAACTAGCCAGGTGTGGTGGCAAACA
CCTGTAGTCCCAGCTACTCAGAAGGCTGAGGTGGGAGGATCACTTGAG
CCTGGAAAGTAGAGGCTACAGTGAGCCGTGATCACACCACTGCACTCC
AGCCTGGGAGACAGAGTGAGACCCTGTCAAATAAATAAACAAACAAAT
AATGATTAAAATAACTAAAACTAATTTTATGCTATTTTCACCTTGTAT
TTTGTAAAGATTTTTAAAATGAAAATTCCCAAATTGCTTTCCAGAAGG
ATTGTTCAAAATTATACCCACATTTCACTCATGTTCTCTTCCTGAACA
GCAGCAATCAGGAAAAACTCCCTGGAAGAGGCAGGGCTTAGACTGAGA
TTTTAAAAGGGGGTAGGCCTCAGCTCTCCTTCCAGGTTTACACTGTGC
ATGTTTCCAAACTCAAAGAATTTACACTCTTCTGGTTGCATTGCTCTG
TAAAGATCTGACCCACTACTATGTATTAAAAAGGGATGCATGATAATG
AATTCAGCCCTCTCTGTAAAATCCAAAGGGTCCTATTGCAGTTTCCCC
CATTTAATGGGTCATTAAAATATTCTTGGGAAGGACAAAGCTTTAGTT
AACTATGAGAAAAACAAGCAGAACCAGCCCTGGATTCTGTCTTCAAAG
ATTTTACCATGTTGGCAGGCCTGGTAGTCCAGAGCCCAAGAAAATATC
CCAGCCACAGATACCCTAGATGTAGACTAGCAGTGCTACAACCTCAAG
GTCAGAAGTATGTCACTAGACCAGAGCCAAAAATAGGTGCTATATCAT
TAAGAGAGTAAAAATGCAAACCACAGACAGGGTGACATTATTCACAAT
AAGCATATAACCCACAGGGGACTCCTATCTGAATATGCAAAGAACTCT
CACTAATCAATAAGAAAAAGGCAAAAGATTTAAACAGGCACTTCACAA
AAAAAGTATATTCAAAAAATCAATAAACATTTGAAAAGATCCTCAATT
CACTAGTTATTAGGGAAAGGTGAAATAAAACCACAATGAGACACCCCC
ACGCCCCCACCAGAACGGCTTAAAATCTAAAACATGTAATACCGAATG
TTTGCAAGGATGCGGAGAAACTGCCATTTTTGTACACTGCCAGTATGA
GGGTAAATCTGTACAACCAGGTTGGAAAACGCTGAGTAGAATGTACTC
TAGCTGGATTTGTGAATATCATATGATCCAGCAATTCTACTCCTAGAA
ATTTACCCAACAGAAATGTGTAAACATGTTCACCAAAAGACACACGCA
AGACAATTCATAGAGGCACTCACTATTCCTAACAGTCAAAAACTGGAA
ACTACCCAAATGTCCATCAGCAGAGAATGGCGATAAACAGTAGCATCT
TCACATAATGAAATGTTTCGACAGCAATGAAAAGTAGCTAGCTACAAC
TACAAACAATGTGATTGAACCTCACAAACATATACTAAGTAAAATTAT
CAGACACAAAGAGTGTATATACTGTATTTAGATACATGTGAAGTCTGA
AAACAGGCAAAACTATTCTGTTGTTAGAAGTCAGAATAGTTACTGCCC
TGCCGGGAAACAGAACTCAAGAGGGCTTAGTAGCTACTGGTAATGTTC
TGCTTCCTGAACTGCATGCTAGTGAGGCAGCTGTTATTTTGTGCAGTC
CTGTGTTACACTGGAGTTAAAAGTTCCCCCAAAATCAGAAAGTGTTCA
GCAAGTGGAAGCAAGTACACTGCTGGACTTGGCTGGGAACTTAGGGGA
TCCCATAATTTGTCACAGGCACAAGCAAAGCCAGCTTTCTTGCCNTAA
GTAGCATCTCCCAGAGTCAGGATCCAGGAATGGTTTGGCAGGCAGGAT
GCAAGGCAGGATTCGGGAGTGGCTGAGAGTTTTCCCAGTGCCACCTGG
TCCCACCTCCCCTCTCCCACTTCTAATGAACGGGCAGTACAGCTTCTG
TTAGGAAAAGAGCCTGGGTCCCTAGGCGATGACTGTCACATCTAGGGA
GAGGGCGATGCACTGGGGTCCTCACCTACACCCCCCTTGGCTGTCTCA
CCACTCTGAATTATAAATGCCCGGACTTCCTCATCTCCCACCCACACA

Fig. 4A 1

TCTTGTTAGAAGAAAAGAAACGAATCTCCCAGGGCTCCTTCTAACAAA
AGTGTTCATTCAGAGTAGCCCTGCTTGAGGGCCCCTGGCCTGGAGGAG
TGGGAGAGGCAGCCCTCCCCCTCCAGGAGAGTCATCTCCAGGGCTACC
CAGGACTGAGTAACTAGGTCACCAGAGTAACCAAAGAGGCAGGAGACA
AGGGCATTCAAGCATTGGGCCAGGAATGGAGGGTGATGTCCAGTTCAT
GTTCTTCTGGTTCCAGCATAGCACACGGTGCAAATGAACCATCATGCA
AGAAAACACAGCTAGTCTCCCTTCCTCCACCAGCAACCTTTGGTTACT
GATAATAATCAAATTCACTATTTTTTTTTTTTTTAACTAAGGCTGAG
ATAATGTCAAAGGACCACAGGGAATAGGAAGGCCTAAACCAAGGCCTT
AAAGAATGAGAAGAAGATTCATTCAAAAAAGCCTCCTAAGGGAGGAAG
ATGTTTTTCCCTCCTTTACTTTTCTACAGTAATTTTTATTTTGGATAA
ATAAACCCTGATAAATGAGAACCCACGCTTTCCCAAGGCCAGGCTGTG
TTTTGGTGGGTGGTCCTCCGTCAGCAGTTGGAGTAATCCAGAGTGATC
CCGGGCAAGTCGGAAGGGAGCAAGTCTGTGTTGAAGCCAAGAGGTATC
TTTCCCTACAGCTTCTCAAGAGAGGGGATCCCCGTGGGTAATTGTGAG
GCTGGAAACACCGAGAGGCTGACTCCCATGTTTATAGAGGTCATTGAT
GGGTTTGTGCATGGAAGGCAGGAGGAGACTGAGAGTGCTTTGTTATTG
TTATTTGGTTTATTTTTATTTTTAAAAAACTGGATCAGCCGACTTTGA
ATACAGAAAATGAAAAATGAGGAGATTTGCATAACAGCGCTTGGACGT
CTGAAGGGGCCCAGGGCCTAGCGGCTGGTGGGGCACCTAGAAACACTT
CTGCCTGCAGATCGCGGAGGGTTAGCCACAGGAAGGGGTCGCCTAGGC
TGGCCACAGGGCCTTTGCTGTGACTGAAGGACCAGCCTTGGCGGCACC
TTCTTTCCCCTCTGCCCTGCACTCCGGCCCCGCCGGAGTCAGAGCTGA
CTTGCTGCAGGTTGGGGAGAGGACAGAGGCTAGGACGGTGGCGAAACC
TCACCTCGTCGCAGTCCGGAAGGTAAACTTGGACCCGGCAGGCACTTC
CTAAAGTCCAAGCTGCCCTCTCTGAAGAATAAACCTGATTTTCCTCCG
GACGCGGACAAAGGAGGATTCGCTCACAACTAGCCTGTAACAAAGATT
CCCTATTTTCGTGGTTAGGAAAAAAAAAAAAAAGGAAGCCCTCCGGGA
GAGACATGCGCCCTAATATTTCTCCCAGATGGGCCGGGTTCAAGCGCG
TTTGAGAGTTTGCTCTCCTACCAGCCTCGGGTTCTAGGCCCCCCGCAC
CCTCATCCTGGCTCCCGCCCCTTCTCTCCACCCTCCCGGACCCCTAAA
GGGGCGGCGGGGCCCAAGCCGAGGGCGCTGCGCCTGACCCCGAGCGGA
AGGGCCCCAGTCTAGGTCCTAATGCGGGTGGCGTCTCCTTTGACAGGC
GGCGTTTGGGGACAACAGCGGGGACGAGAGATAAGGTGACATACCAGA
GCAGATTTGGTGCGCGCGCTGATACTCCTCTCCCGACAGGAAACGCGG
AGCTATTTAAAAGACCCTATCGATTACTTTATCTTTCCTGGAAAGCTT
CTTGCGGAGAGACAAAAGATGTTCCCTGCCTAAAGACACAAGGCCACA
CAACGGAGGGTCTGCACAGGCGACGCACAATTCGGCGCGGGGAAAGCA
AAAACACACTGACGCTTAGAGTGCACAAACGTGTGTGTTCCCAGAGCA
GCTCCAGAGTGCGGCAGGGACGCTGGGGGCGGCGAGGGGCACCCACAG
TATGGTCTTCTGTGCCCTTGGAAAGTTTTTTTTCACCGTATGCGCGTA
AAACACGCACACACAGAGAAAGTGACTGTGCACTTAGGGCGCCTGTGT
GTACCCGTGTCGTTTTAGCGAATTTAAAGCACATCAGGCCGGGCGCCA
TGGCTCACGCCTGTAATCCCAGCACTTTAGGAGGCCGAGGCGGGCCGA
TCACCTGAGGTCGGGAGTTCGACACCAGCCTGGCCAACATGGTGAAAC

Fig. 4A 2

CCTGTCTCTACAAAAAATACAAAAATTAGCCGGGCATGGTGATGCGTG
CCTGTGATCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCGCTTGAA
CCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCACACCACTGCACTCC
AGCCTGGGCGACAAGAGCGAAATTCCGTCTAAAAAAATAAAATAAAAT
AAAATGATAATTAAGCCCATCAACTCACATTCAAAGCGGTTACTGGTG
GTTGTAATGTATCCATAGACACAGGTCTAAAATGTAAACGCTCCATTG
TGCTCCTTTTAAGGGCTTGAATGTCTGCAACTGTCATGTGTACACTTA
AAGTATGGGATGTGTCAACACGACCCTTTCTAGCGCGCTCGTTTCGTG
TCTGAATCCCCGCATTTCGCCAATTTGCTTGGAGCGCAGAACGCCCTC
CGCGAAAGGCGGCTGCTGATCCCGACTTTGCTCCGGTATCGCGCAGCT
TGTTGGCCTCCGGGTCCCCCGTGCCATGCCCCCGGGAGGCTCTCCACA
GACACCGCTTGCGCCGAATTATACGAGACTGAATGGGTTTTTTGGTG
TGTGTGTGCAACACAACAATTTGTCAGCTGCTGTTCACAATGCGCTCC
GCCGGGCGGTGGAAACTTGGCTGCGGTAACGCACAGCAGGTTGGAGGG
CACGACCCGGAAGGAAGGAAGAGGCGAGGAGGGAAAGGCGGCGACCCT
AGGCCCGCTGGCCAGCCGTTTCCAGCATCAATTCAGCACTGAGCCGGC
CGCAGCAGCACAGGGCTGGGGGCTCCCGGAAGTTCGGCCAGCCGGGGT
TTGGGCCAGAGCCGCGGAGGCTGCCCGGTGGTAGGTGCGACTCTTCAC
CTCTCCGGGGAGCGGCGGCCGACGACCCAACCCACCCGCAAGCGCTGC
CGTCGGCCCGGCTGGTCCCCCGCGCGGGCACAAAAACAGGCGGCAGTT
CGCCAGCTCTCTTTTCCCAAACCTGAACCGCCAAGCCGAAGGTTCTTC
CAAAGTCGCGGTTCCCCGGGCTTCACACCCGCCGGGCAGGCGCGAACC
AGCCCCAGGACAACCATTTTCCTCTTCACTGTATCTGAGTCGTTGTCC
ATCTGACTCGAATGTCACCTGATTTTCCCAGCTGTGACCTCCAGCGAC
GGGACTCCGAGGAACTGATTCCAGCGTCTCGATTCTCTCCGCCTCTCC
GCCCGTTTTGGCTGAAGCGGTTTGCAGCCGTCGGGGCAGAAGGGGTGG
GATGTGGCAGCCACCAGCCCCAGCCCAGAGAAGAAAAGAGGACGAAAT
TAACGCGAAAGGACACCGGAAGTCTGAAAGCGACTCCCTCGGATCCTC
GGAATCCGAGGCAAACCCTAACACTAGTTTGAAAGCGGATCATATCCA
CTAATCCAGGACAAATTCGGGTTGGGAAACATACTCCCCAGAGCCTAA
GAAAACTGACTTACAACAAAACAAAACTGACAAGGACAAAATGCAAAG
GAGTTTGTGAAACGTAATTGCTCTCAGAAAATATGTGTATATATATAC
ATCCTATAATATGTTTTAAATTTGCAAAAAAAAAGTCTCTAAGAGGAT
ATATTTTTAAAACCAGTGGCAGCTTGGGAGGGAGTGGGGATTAGCTGA
GAAGGGGAGAAGGAAGCATTTTTGAGGTGACGTAAATGTTTTTGTATC
TTGATTATGGTGGCTGTTATGGGGGTGCACATCCAAGTGTCAAGACTC
ATCGAACTGTACACTTTTGTTCTAGGTACATTAGACCTCAATAAAGTG
GATTTTAAACCTAAATAAGCCAGGTAACAGCTTTGCCTGGGTGGCTGG
GGGAGAGGCTTGGGACACTTTACATTGATCTCCCTCTTAGGCATGTTC
GTTTTGGTTTGGTTTTGTTCTTATGATGTATTATTTATTCAAAAATAT
ATCATTAGCAGAGTGACTGATGTAAATGTAAAACCATTGTTAAGGAAA
CCAACAAAAGCGGGAACAAGAGACACTGGTGCATCCTGTTAGAGGGAT
AAGAATAAGCACTCGCTGTCCAAGCTCATAAAATATTTTGGGAATGAA
TGTCGTTCCGCTTTGTTTTTTTGGTTTTTTTGCTCATGTGTTTAACAT
CAACGAGAAATGAGGACCCAAAACTTATCCAGTGGTTACGTGTGGTGT

Fig. 4A 3

GTGTGGCTGTCATCTCCTTGGGACTGGCTACTGAAGGCCACAGGCGTG
GGAGGACCAAATGCTCCCTGGATGTTGAGTCCCAGCCGGTAAGCAGCA
CACAGTCCCGCTTGCAGCAAAGATGTGGTGGCCGGCTGCGCTGTGGGG
GAAGGCCAGGCCCGGACAGGAACCTCAGATCTCACCGGCGGATGAGAG
TGGTGCCCCCTGCAGCTGGAGTCCCTGCTGGCCTGAGAGCTCCAGCTG
TGCCACCGTTGGGCAGACCCCACACTTCAGGGAGCTGCCAGGATCAGT
GGCTACAAGAGTCCCCACCGTGTTTGGAGAAACTAGGTATGAAATATT
TCCATTTACACCCCTACCCCGGCCCCAGACAGGAAAGTCACTTCAACC
TTGTTAGGTCAGATTCCAGATCTGGTTCAGATGCAGGGCTATTTCAGA
GAGATTTTTAGAGGCTGACTCTCAGGAGAGGGAAGGACAGTGGGCTGA
AGGCCAGGGGTCAGGAAATCTAGGAACTGCTAAACTCCTCTGCTGGCC
TGCGGGGAGCGCCCGGGTGGGGCTACCAAGGCCACAAGCCAGTTCCAT
CTTCCCACTTTGCCACCTTCTCACAGGGACCAGGCTCTGCATCCTCAG
TGACCACAAGACTTGGGCCTGCCCTCTAGTTTGTCTATACCTGCCCCC
TCCCTTGACTCATACTGTCCAAGACCCCAAGACCAAACCACAAGTCAG
GAGAGATCTTGAGGGCAGCCAGTGCCACCAGGGTCCTGTTCCCAGGTA
CTACTAGACAAAGGCCACCCTTCCTCCCCTCTCTCTAGGGCTCCGCTG
ACCACCCTGCACAGTCTTCCTACACCAAGGGCTCCGGTGCCACCCCTT
CACAGAGAGTTCACTGCACCGCTGCTTCGGCTGCCTGTCTCAAACCAT
ACACACACCTTTGATTCTTAAACTCCAAGATTAGGATGGGCCCCAGAA
ATCTGCATTTTTAATATGTACCTCAGAGGATTCTGGCCTAGATATTTC
TACAGCCCCAAAAGTAACAAGGAACCTGTTCCAAAAAGTGTATTACGG
AAACTGTCATGTTTATTCTTGACTTGCCCCCCAATTATTCTTCCCCTG
AAGTTTTCATCACCAAAAAACCCCACATGTGAACCATATGTGTACATA
TGCCCATATTTAAAATACAAATTCTGCACCTGGTTTGCTATTTAAAGT
ATCTCAAAACATATCCATAAGAATACATATGAATGGAACTAATTCTTT
CTCATGGGATATGGGATCTGTTCTATGGACAACATAATTTTTAACCAG
TCCTAGTATATATACACTGGTTTTTACATGTTGATCTTAAAAAATAA
AAACGGNTGAAA (SEQ ID NO.: 4)

Fig. 4A 4

CAATTTCTATTNAGTTCTATTAAAAGGGATTTTTTTNAACTCACTGGNAACCAGGAGGA
CTGNAAAGAAAAGTGAAATGGCTCTGGGACTTTCCTCTAAGGAGACCAGCATGGGTCGCC
CCAATTTTTATTTTGCACGTATTTGTCCGTTTTTGCCCCATCTCCTCTCTCCTGAAACAC
CAAGACCTTTTTGGAAGCCAAGAGAAATCATTACCCGATTCACAAAGAGCATAGAGAGTG
TAACAGTCACTGATCTTGTTCAAATAGGGAGAGTTTTTTTCCTTCCCTTTTTGTAACAC
CTGACCCACAGGACTGACAGTTCTAGGAAGCCCCCTTACCCGAAAATAGGAAATAAATCC
TTGCCACCTTGATTTGCAAGGGCAATGCTAATTTTTTTCTTTCTCCAGAGCTCTCAAAAA
AAAAAAAAAAAAAACCTTACTAAAAACAGGGATCCCGGATGTAGCCTCGATGTCCCCCAT
TAAACGGTAATATTTCAGGCGTCCGCTCACACTAATCTTTCAAACTGTCATCGCGAGCCG
CCTGGCCAGCAGATTCACTTAACAGCGCTCCCAGGACCCTCGTTCCGAGCTCTTTTCAGC
GAGACATTTAATTGAATCGGATGTGGCTCGTTTGCCAGACGTCACCGCCTCGGCGATAGG
CATCCTCTCCAACGACACCCCCCCCCGCCCGCGCTCGAAAACAATCTTCAAAAGGCAAGG
GGGCCCCCCAAGTAGGTTAATTTACAACCATAACGGTAACGTGGCCAAAAGNCAGGCGAG
GAAGGGCCGCAAGGCCGCTGACATGCAAGCTCCGTCCAAGAAGAATTTGGGTTGGAGGTG
AAGAGGTGGGGGGACGAGGTTTCNTGGGCCTTGAACGCCCCACATTTAAAAAAGGCATCC
TCCACAGACTAGACTAACAATTCCAGACCCCCAGTAGTCCCTGGCTCAGAAACTCGAGGC
GTGATTTCGGCGTGGCAGCCCAGGCCTGTTACTGACGGCTGGCGCCTAGAAGCCGGGGTC
AGGGCGTTGCGCGCCTCCTGGGCTGCCCTGCGGGGCTCACCTCTCTCCCCAGCATGGAGG
CCCCAGGTCCTGGGAGTGTGGCTTTGATGAGGGACAGGAAAAGTCCCAACATCAGGCCAA
TGCTTGACTTCACTTGCGTCGGCGTCTCAGACGGCACACTGTCGGGTTTGAGCACCCAAG
ATGTACGTTCTGGACAGACACTATTTTGTCCCCATACATGGAGCGTTTCCTCCGCACCTT
GGGCGCGCCTGCGGGAGCTGTGTCTTTAGGTAGTTTTTGGCCCTGCGCCGCCTTTATTCT
ACTCCAAGCGCTCTTTGCCAAACCCGCACTCCGCAAAGAGCCAAGCCCTCCACATCCCCA
TTCTCAGCAAGTCCACGCGTCCCGCCCAGCTTCCCGCCCGCGGTTCCCTGTACCAGCTAG
GGCCGTGAGAAGCCAACGCTTTTCCACTGACAAATCCTGTCATCCCCAGCTCTAGAAGGC
GTCCTTAACCTGGGCCCGCTCTGCCTGCCCGGACTCCTGAATTGTAAGCAAAATAAAACT
CCTCTCTGCAGTGTTCTGGGGAATGGAGAAGACCCCAAGCTTTCATCAGACCCTCCCAAG
GAGTGCGGGGACCCAGAGAAATGAGGCCACCCGGGCAGGATCTGGCCATGTAGCTGGCGC
TCCTGAAACTCTGGCAGATTTGTCTGACTTCTGTGCCCTACTCTACTGACCCTGGGCTAA
AAATGATCATGATCACCCCACTTGCCCTGCCCTTCCCCCACGCGCCTGACCGAGCCGCAG
GGGTGCCCCACTGGAAGTCCGGCCCAGAGGCCTCAGAGAAATCCTGGCCTAGCTGGGCTC
AGAGGAGCCCCGCCTCCCTGAGAGCTAAACCTGGGCTAGGACCCTGAAACCTCGAGGTTG
GCAGAAGCCTGAGGGCCTTGCTGCCAGGCAGGGAGGGCACGGGAAGGAGGGAGGTGGGAT
CGATGGCCTCCAAACAGGGGAAACAAGGTGGCTGGTAGCTGGGGCACTCCACAAGACAGG
TGTNTCCTGGGAAGCTGAGCTTACCAGCTGGGATTCCTGATTTATTTCATTATTAAGGGG
AGAGGCATTTCCCCTGGGAGGGTACTGGCAGTGACTGATGCCCCCTGGAGTTGTGCTGTG
CATAACACTACTGTAGGAGGCAGCAACTCCTACCCCACCTGGCCATCACTCACCTTGCCC
TTACTTTCGTTGATTCGCCCAGAAGCACCCAGAGCCTGCGGCATGATTGACCCTGTAGGC
CAAGCCAAACCAAACCCCCGAATTGTCCAGAATTTTCGCCCTGGTGTATCCCCAAAGCCC
AGCCCTGTCTTTNAGGGTTTTTTTCCTATTGAGATTTTCCCTCATCCCACCACCTTTAGT
AATAAAGCCTTCCTCAAACTAATTTCCTCCCCACCGCTTCCCACCCCATCCTTTTTTTTT
CCCATGCTGGTTTGGGTGCTGAGGAATATTTTTTCAAACCCACACCCATCCAGCCCTGCC
CAGAGGCCTGACTTTGCATGCCTCTGGTAGGNTTTTCAGGGTTACATTAGGGAGCAAAAG
CAGGGTGCAGGGGCAAAAGGGGACCCTTCCAAATGGGTCGTGGCCCCTTTAAAAAAGCTG
GGCAGGGNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCGTATGACTATA

Fig. 4B 1

TTAGGTGACACGAAACTGCTCATCGCTCCTGTCATCGAGGCCCCTGGCCCAATGGCAGGC
TGAGTCCCCCTCCTCTGGCCTGGTCCCGCCTCTCCTGCCCCTTGTGCTCAGCGCTACCTG
CTGCCCGGACACATCCAGAGCTGGCCGACGGGTGCGCGGGCGGGCGGCGGCACCATGCAG
GGAAGCTGCCAGGGGCCGTGGCAGCGCCGCTTTCTGCCGCCCACCTGGCGCTGTGAGAC
TGGCGCTGCCACCATGTTCCCCAGCCCTGCTCTCACGCCCACGCCCTTCTCAGTCAAAGA
CATCCTAAACCTGGAACAGCAGCAGCGCAGCCTGGCTGCCGCCGGAGAGCTCTCTGCCCG
CCTGGAGGCGACCCTGGCGCCCTCCTCCTGCATGCTGGCCGCCTTCAAGCCAGAGGCCTA
CGCTGGGCCCGAGGCGGCTGCGCCGGGCCTCCCAGAGCTGCGCGCAGAGCTGGGCCGCGC
GCCTTCACCGGCCAAGTGTGCGTCTGCCTTTCCCGCCGCCCCCGCCTTCTATCCACGTGC
CTACAGCGACCCCGACCCAGCCAAGGACCCTAGAGCCGAAAAGAAAGGTGAGGAGGAAAC
ACAGGCCCCCTTCTCCCCTCCTGGGTCGCTTTCGTCCCCAAGAAACTCAGGGCCAGGAGG
AGGACACACGCGCCCTTGGGCCGAGGGCTGGGCTGCGGCGGGGGGTTCAGAATGTAAGAT
GCCTGGTGTTGTCGCCAGGCTCCCGCGCCCCGCGTCCAATCGGAGGTTCAGAGGAAATGC
CGGATTGAAAGGATCCGAAAGCAAGAGACCAAAAAACTTTTCCCCCCGGCCTAACAAACC
CCCGGCGGTTTCCGCTCTGCTCCTGGTTCTGGTAGAATTTTAAAAATCGGTTTATGGTTA
AACAAAACAAAAAAACAGCCAAAACCCCCGTTTTTACCCCCCCCTTGGATTTTCAAACC
CTTTTTAAAATTTTTGAAAAAAAACCCCCAAACAAAATTAAATTTTTTCCCCCAAAAAAT
TTTTTTTTTTAACAAAAGGGGGGGTGGAAAATTTTTTTTTCCCCCCCCCAAAAGGGGTT
TTTGTTTTTTTTTT--------TTTNTTTGGCAAAAATGAATTNTGGANCNAGGCCTTAT
TTNAAATGGATATTGGGNCCNCAGGATTTTGATTTCATTTATTTTTTTAAGCAAACTTNC
CGGGCCGGCAAGGGGAAAGGTTCCCTCGTGGAAAAGTAGGAAATGCTGCGCTACCGCGGG
CACAAGGNAGTGGACGAGATGAGTGCGGGATCATCCCGCAGGCCATCCCAGGATCGGGGA
GGGAGGCCGGCCCCGCTGCAGAAAGGGGCTTCTGGGAGACCCCCCAGCCCAAGGCAGGAG
CCCGGGCGATTCCCGGGAGGCCGCAGGCGCTGGGCGAAGCGCTGGGCGAAGGGCCGCTGC
CAGCCGGGAGAGAATTCATAGGTTTGTTGAGGAGCAGAGGCCTGGGAACAAATTCGGGCG
GGCACGGCGGCTAGAACTGATCGCTACCAATTCGAGGAAGCCAGCAAGGCAGGTTCCGAG
GCCGCCTGCCCACCCGCAGCTTCTTGGACACTGCGCAAACCCTGCTGCGGCCAGGCTGGA
GCCTCCGATCACCAAACCAACACTCCCTGGCCTTCTGTTTCTTGATTCCTTAATTTTGAG
ATAAGACCGTCCCTAGCAGTGAGGCCTCGGCCTCTGTTCATTTAACTTCTCAAACCAAAC
TAGCCCTAATTCAGTTCACCCCAGAGCATCACCTGGTTTTATTTTTATTTTTTTATTTTT
TTATTTATTTTTTTTTTTTTTGCAGCCTGAAATTTTAAGTCACCGTTTGTCTCCCTCACC
AGGGTGTGAACTGCCCCGAGGGCAGAGACCTCCCGTTTTGTTTTCCAGCGCCTTGAGCCA
GCTTGACTTTTTACAAATGCTGAGTGAGACGTGTCGGTGGCTCCCAGTGCACTTGGCAGA
GTGAGCCGCAGCCAGCTGGGCGCTCCAGGCAGGACACAGTGGCCTCCACGAGGATCCCTT
ACCATTACTGTGCGGCCGCGCTCCGTAGGTCAAGCCGCTCTTACCAAGCGTCTTTCTGCC
TTTCTGTTCCCCCTCAGAGCTGTGCGCGCTGCAGAAGGCGGTGGAGCTGGAGAAGACAGA
GGCGGACAACGCGGAGCGGCCCCGGGCGCGACGGCGGAGGAAGCCGCGCGTGCTCTTCTC
GCAGGCGCAGGTCTATGAGCTGGAGCGGCGCTTCAAGCAGCAGCGGTACCTGTCGGCCCC
CGAACGCGACCAGCTGGCCAGCGTGCTGAAACTCACGTCCACGCAGGTCAAGATCTGGTT
CCAGAACCGGCGCTACAAGTGCAAGCGGCAGCGGCAGGACCAGACTCTGGAGCTGGTGGG
GCTGCCCCCGCCGCCGCCGCCGCCTGCCCGCAGGATCGCGGTGCCAGTGCTGGTGCGCGA
TGGCAAGCCATGCCTAGGGGACTCGGCGCCCTACGCGCCTGCCTACGGCGTGGGCCTCAA
TCCCTACGGTTATAACGCCTACCCCGCCTATCCGGGTTACGGCGGCGCGGCCTGCAGCCC
TGGCTACAGCTGCACTGCCGCTTACCCCGCCGGGCCTTCCCCAGCGCAGCCGGCCACTGC
CGCCGCCAACAACAACTTCGTGAACTTCGGCGTCGGGGACTTGAATGCGGTTCAGAGCCC

Fig. 4B 2

CGGGATTCCGCAGAGCAACTCGGGAGTGTCCACGCTGCATGGTATCCGAGCCTGGTAGGG
AAGGGACCCGCGTGGCGCGACCCTGACCGATCCCACCTCAACAGCTCCCTGACTCTCGTG
GGGAGAAGGGGCTCCCAACATGACCCTGAGTCCCCTGGATTTTGCATTCACTCCTGCGGA
GACCTAGGAACTTTTTCTGTCCCACGCGCGTTTGTTCTTGCGCACGGGAGAGTTTGTGGC
GGCGATTATGCAGCGTGCAATGAGTGATCCTGCAGCCTGGTGTCTTAGCTGTCCCCCCAG
GAGTGCCCTCCGAGAGTCCATGGGCACCCCCGGTTGGAACTGGGACTGAGCTCGGGCACG
CAGGGCCTGAGATCTGGCCGCCCATTCCGCGAGCCAGGGCCGGGCGCCCGGGCCTTTGCT
ATCTCGCCGTCGCCCGCCCACGCACCCACCCGTATTTATGTTTTTACCTATTGCTGTAAG
AAATGACGATCCCCTTCCCATTAAAGAGAGTGCGTTGACCCCGCACGTGTGCTTCTTTCA
GCTTGCGGCGCTTCAGAAGCAGGAGAGAGGTGGCCGCCCGGGACTGGTCTCAGATCTCAG
GCACAGGCATTCCCTGAGCAAATTGATAACATTGATACTAATAAAACCTAACCCTTGCTG
GAACCATACTGGTTCCGTGTCGGGCACTTTCTGAGATTGTCTCATATAATCCTCAATAAT
CCAAAAAAAAAAAAATCCTAAAGTTTAGAAGCTGAGGCCCGGAGAGGTTTAATGACTTAC
CTGCGAGCAAATAGCCAGTACTAGTCGAACTCTGGTTAAATTCAGGATGCCTCACTTCAG
AGACCGCCTTCCCTGTGCTCCCAAGCTCCCCTCCTTGAATCCTAATGTGTGCCAGGCACG
GTTCCAGGCACTGGGCATTAAATGGACAAGCAAAAGAACCTGGGCCCTCTGTAGCTGGAG
AGCACCGTGATCATCCCACTTAAAAGAACTCCTTAACCTGTTTCCAAGATGGNAAAAGCC
AAGAANCCAAAGCCCTTGGGNAAGCGTTCTCAAGGGTCCTCANATGCCCCAAATGCCACG
TCGGGGGCTCAACANCTNGCCCGTTGGAACTGAATGCCNANGGTGGGCCCCAAANAAGGN
TCCTGCGGGATGGNGCTCAACTCCAAGCTGTGGTGAAGGCCCATAAAATTCAAATGGGCC
AAGGGGAGCCCCCTAAAGCCCTAAACCTTCNGGGGGTCCNTTCCCTAAGGGCATTTAANT
TTACCAAAAGTTTGGNCAAANAATGTTTCCAATGGNCCNGATTTTATNGANGGGNAAAAC
TGGNGGGCAACCGAAATCCAGTTTAAACCCGGGTTGTTT (SEQ ID NO.: 5)

Fig. 4B 3

AGGCCCCCCG CACCCTCATC CTGGCTCCCG CCCCTTCTCT CCACCCTCCC
GGACCCCTAA AGGGGCGGCG GGGCCCAAGC CGAGGGCGCT GCGCCTGACC
CCGAGCGGAA GGGCCCCAGT CTAGGTCCTA ATGCGGGTGG CGTCTCCTTT
GACAGGCGGC GTTTGGGGAC AACAGCGGGG ACGAGAGATA AGGTGACATA
CCAGAGCAGA TTTGGTGCGC GCGCTGATAC TCCTCTCCCG ACAGGAAACG
CGGAGCTATT TAAAAGACCC TATCGATTAC TTTATCTTTC CTGGAAAGCT
TCTTGCGGAG AGACAAAAGA TGTTCCCTGC CTAAAGACAC AAGGCCACAC
AACGGAGGGT CTGCACAGGC GACGC (SEQ ID NO.: 1)

TGCTCCTTT TAAGGGCTTG AATGTCTGCA ACTGTCATGT GTACACTTAA
AG (SEQ ID NO.: 2)

Fig. 5A

AGGCCCCCCG CACCCTCATC CTGGCTCCCG CCCCTTCTCT CCACCCTCCC
GGACCCCTAA AGGGGCGGCG GGGCCCAAGC CGAGGGCGCT GCGCCTGACC
CCGAGCGGAA GGGCCCCAGT CTAGGTCCTA ATGCGGGTGG CGTCTCCTTT
GACAGGCGGC GTTTGGGGAC AACAGCGGGG ACGAGAGATA AGGTGACATA
CCAGAGCAGA TTTGGTGCGC GCGCTGATAC TCCTCTCCCG ACAGGAAACG
CGGAGCTATT TAAAAGACCC TATCGATTAC TTTATCTTTC CTGGAAAGCT
TCTTGCGGAG AGACAAAAGA TGTTCCCTGC CTAAAGACAC AAGGCCACAC
AACGGAGGGT CTGCACAGGC GACGCACAAT TCGGCGCGGG GAAAGCAAAA
ACACACTGAC GCTTAGAGTG CACAAACGTG TGTGTTCCCA GAGCAGCTCC
AGAGTGCGGC AGGGACGCTG GGGGCGGCGA GGGGCACCCA CAGTATGGTC
TTCTGTGCCC TTGGAAAGTT TTTTTTCACC GTATGCGCGT AAAACACGCA
CACACAGAGA AAGTGACTGT GCACTTAGGG CGCCTGTGTG TACCCGTGTC
GTTTTAGCGA ATTTAAAGCA CATCAGGCCG GGCGCCATGG CTCACGCCTG
TAATCCCAGC ACTTTAGGAG GCCGAGGCGG GCCGATCACC TGAGGTCGGG
AGTTCGACAC CAGCCTGGCC AACATGGTGA AACCCTGTCT CTACAAAAAA
TACAAAAATT AGCCGGGCAT GGTGATGCGT GCCTGTGATC CCAGCTACTC
GGGAGGCTGA GGCAGGAGAA TCGCTTGAAC CCGGGAGGCG GAGGTTGCAG
TGAGCCGAGA TCACACCACT GCACTCCAGC CTGGGCGACA AGAGCGAAAT
TCCGTCTAAA AAAATAAAAT AAAATAAAAT GATAATTAAG CCCATCAACT
CACATTCAAA GCGGTTACTG GTGGTTGTAA TGTATCCATA GACACAGGTC
TAAAATGTAA ACGCTCCATT GTGCTCCTTT TAAGGGCTTG AATGTCTGCA
ACTGTCATGT GTACACTTAA AG (SEQ ID NO.: 3)

Fig. 5B

AGAGAAATCA TTACCCGATT CACAAAGAGC ATAGAGAGTG TAACAGTCAC
TGATCTTGTT CAAATAGGGA GAGTTTTTTT TCCTTCCCTT TTTGTAACAC
CTGACCCACA GGACTGACAG TTCTAGGAAG CCCCCTTACC CGAAAATAGG
AAATAAATCC TTGCCACCTT GATTTGCAAG GGCAATGCTA ATTTTTTTCT
TTCTCCAGAG CTCTCAAAAA AAAAAAAAAA AAAACCTTAC TAAAAACAGG
GATCCCGGAT GTAGCCTCGA TGTCCCCCAT TAAACGGTAA TATTTCAGGC
GTCCGCTCAC ACTAATCTTT CAAACTGTCA TCGCGAGCCG CCTGGCCAGC
AGATTCACTT AACAGCGCTC CCAGGACCCT CGTTCCGAGC TCTTTTCAGC
GAGACATTTA ATTGAATCGG ATGTGGCTCG TTTGCCAGAC GTCACCGCCT
CGGCGATAGG CATCCTCTCC AACGACAC (SEQ ID NO.: 6)

Fig. 5C

Transgenic Constructs of the Human Csx/Nkx2-5 Enhancer

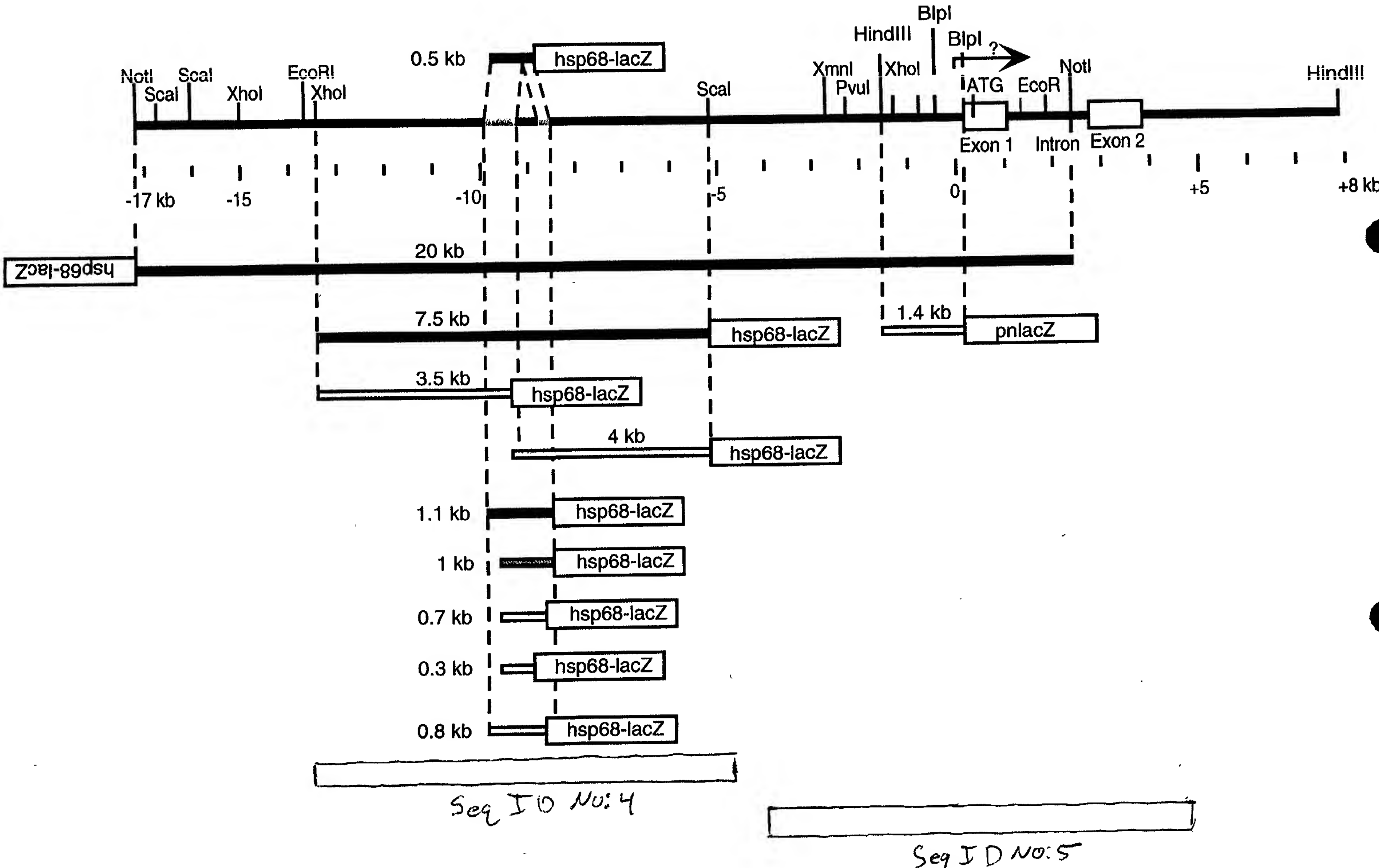


Fig. 6

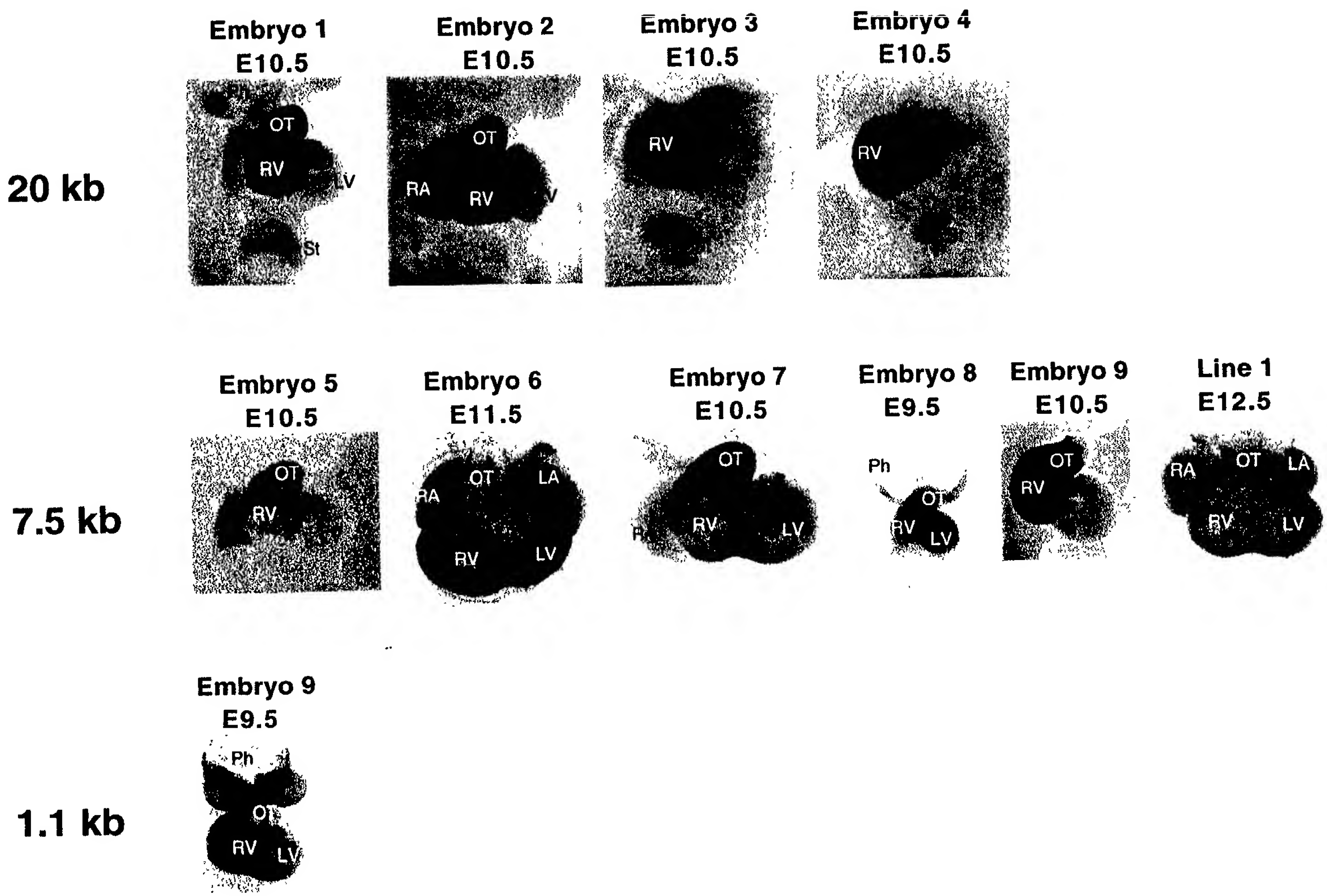
Transgenic Analysis of the Human Csx Enhancer Sequence

Constructs	# of Transgenes	Enhancer positives (Cardiac : Ectopic)[1]
20 kb	8	4 : 0
7.5 kb	8	6 : 1
promoter-proximal 4 kb	7	0 : 1
promoter-distal 3.5 kb	6	0 : 0
1.1 kb	8	3 : 1
1.0 kb	10	1 : 2
0.7 kb	8	0 : 3
0.3 kb	11	0 : 6
0.8 kb	6	0 : 1
0.5 kb	2	2 : 0

1. Each embryo was classified into either 'cardiac' or 'ectopic' judged upon the extent of similarity to the endogenous Csx expression pattern.

Fig. 7

Cardiac Expression of the hCsx Enhancer-hsp68-lacZ Constructs

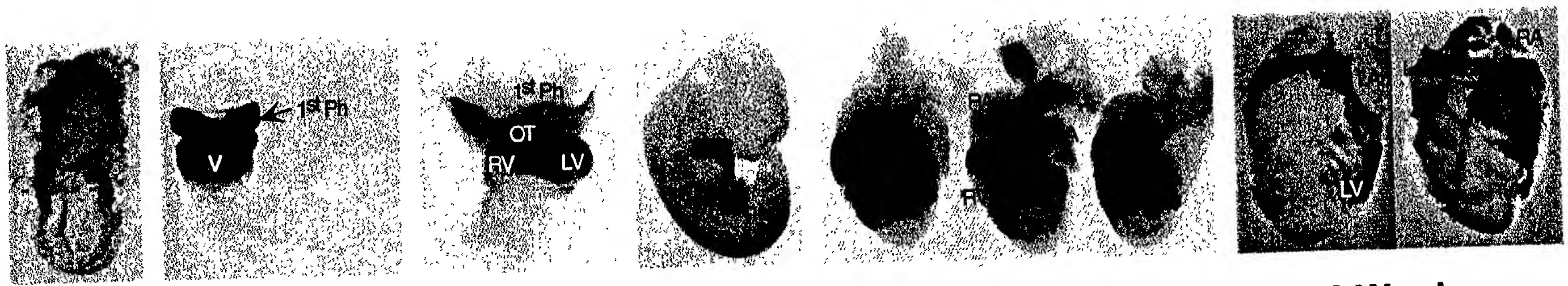


RA; Right Atrium LA; Left Atrium RV; Right Ventricle LV; Left Ventricle

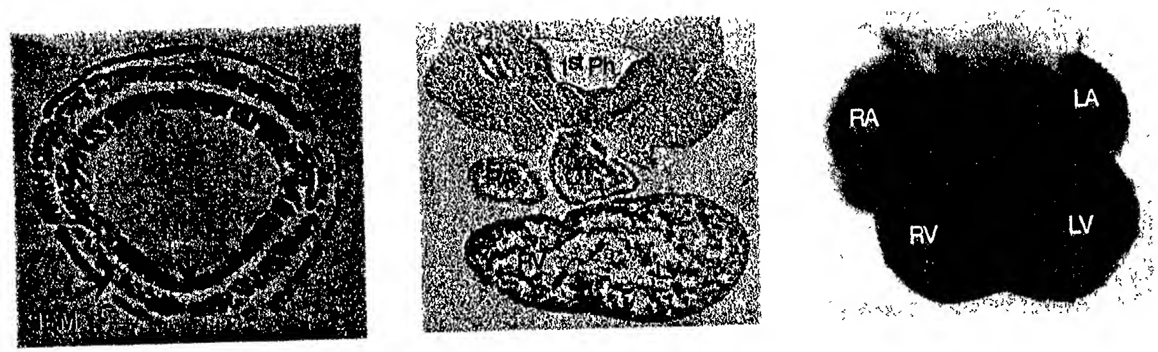
Ph; Pharyngeal Arch St; Stomach OT; Outflow Tract

Fig. 8

Cardiac Expression of the 7.5 kb hCsx Enhancer-hsp68-lacZ Construct



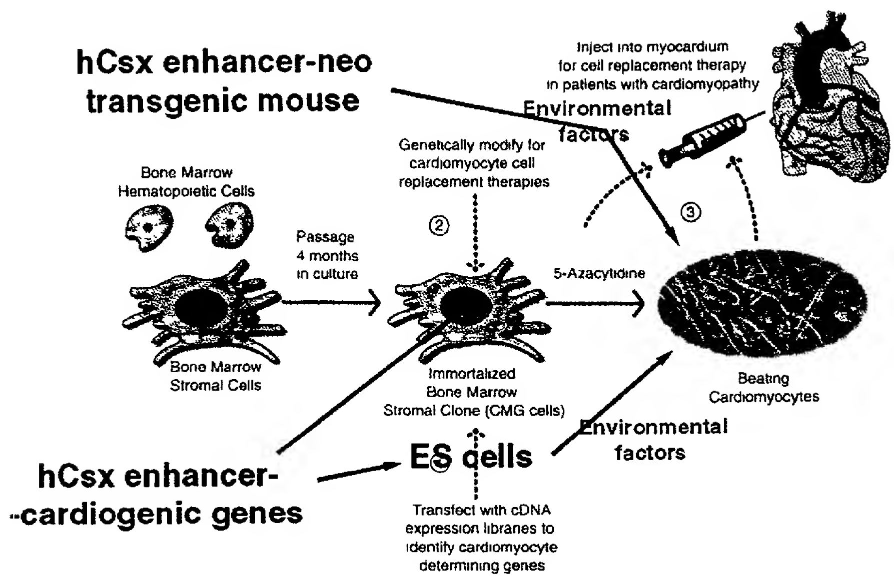
E7.5 E9.5 E10.5 E12.5 3 Days 4 Weeks



E7.5 E10.5 E12.5

RA; Right Atrium
RV; Right Ventricle
OT; Outflow Tract
IEM; Intra Embryonic Mesoderm
NG; Neural Groove
LA; Left Atrium
LV; Left Ventricle
Ph; Pharyngeal Arch

Fig. 9



Facilitated isolation of cardiac myocytes.
Modified from [J. M. Leiden, JCI (1999) 103:591]

Fig. 10